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(71) Applicant (*for all designated States except US*): MILLENNIUM PHARMACEUTICALS, INC. [US/US]; 640 Memorial Drive, Cambridge, MA 02139 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): TARTAGLIA, Louis, A. [US/US]; Apartment 6, 104 Coolidge Hill Road, Watertown, MA 02172 (US). TEPPER, Robert, I. [US/US]; 53 Laurel Road, Weston, MA 02193 (US). CULPEPPER, Janice, A. [US/US]; 1734 Beacon Street, Brookline, MA 02146 (US). WHITE, David, W. [US/US]; 393 Pine Street, Holbrook, MA 02343 (US).

(74) Agent: FASSE, J., Peter; Fish & Richardson P.C., 225 Franklin Street, Boston, MA 02110 (US).

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(54) Title: THE OB RECEPTOR AND METHODS OF DIAGNOSING AND TREATING WEIGHT

(57) Abstract

The present invention relates to the discovery, identification and characterization of nucleotides that encode Ob receptor (ObR), a receptor protein that participates in mammalian body weight regulation. The invention encompasses obR nucleotides, host cell expression systems, ObR proteins, fusion proteins, polypeptides and peptides, antibodies to the receptor, transgenic animals that express an obR transgene, or recombinant knock-out animals that do not express the ObR, antagonists and agonists of the receptor, and other compounds that modulate obR gene expression or ObR activity that can be used for diagnosis, drug screening, clinical trial monitoring, and/or the treatment of body weight disorders, including but not limited to obesity, cachexia and anorexia.

CLAIMS:

1. An isolated nucleic acid molecule containing the nucleotide sequence of obR gene.

2. An isolated nucleic acid molecule which encodes an Ob receptor, or a fragment thereof, having a nucleotide sequence that:

encodes the amino acid sequence shown in FIG. 1 or the amino acid sequence encoded by the cDNA contained in cDNA clone famj5312 as deposited with the ATCC having accession No. 69952; or

encodes the amino acid sequence shown in FIG. 6; or

encodes the amino acid sequence shown in FIG. 3 or the amino acid sequence contained in cDNA clone fahj5312d as deposited with the ATCC having accession No. 69963, or in genomic clone h-obR-p87 as deposited with the ATCC; or

hybridizes under stringent conditions to the nucleotide sequence of (a), (b) or (c) or to its complement.

3. An isolated nucleotide sequence encoding a polypeptide corresponding to the extracellular, transmembrane or cytoplasmic domain of the Ob receptor protein, or a deletion mutant of the Ob receptor protein in which the transmembrane domain or the cytoplasmic domain is deleted.

4. An isolated nucleotide sequence encoding a chimeric protein comprising the polypeptide of Claim 3 fused to a heterologous polypeptide.

5. The isolated nucleotide sequence of Claim 4 in which the heterologous polypeptide is a constant region of an immunoglobulin.

6. A nucleotide vector containing the nucleotide sequence of Claim 1, 2, 3, 4 or 5.

7. An expression vector containing the nucleotide sequence of Claim 1, 2, 3, 4 or 5 in operative association with a nucleotide regulatory sequence that controls expression of the nucleotide sequence in a host cell.

8. The expression vector of Claim 7, in which said regulatory sequence is selected from the group consisting of the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage λ, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α-mating factors.

9. A genetically engineered host cell that contains the nucleotide sequence of Claim 1, 2, 3, 4 or 5.

10. A genetically engineered host cell that contains the nucleotide sequence of Claim 1, 2, 3, 4 or 5 in operative association with a nucleotide regulatory sequence that controls expression of the nucleotide sequence in the host cell.

11. The genetically engineered host cell of Claim 10 in which the host cell is a fibroblast, a Chinese hamster ovary cell, a COS cell, or a VERO cell, a hypothalamic cell or a choroid plexus cell.

12. An isolated Ob receptor protein.

13. An isolated Ob receptor protein having the amino acid sequence shown in FIG. 1, 3 or 6, or the amino acid sequence encoded by the cDNA contained in cDNA clone famj5312 as deposited with the ATCC having accession No. 69952, or the amino acid sequence encoded by the cDNA contained in cDNA clone fahj5312d as deposited with the ATCC having accession No. 69963, or the amino acid sequence encoded by the genomic clone h-obR-p87 as deposited with the ATCC.

14. A polypeptide having an amino acid sequence corresponding to the extracellular, transmembrane or cytoplasmic domain of Ob receptor protein, or a deletion mutant of the Ob receptor protein in which the transmembrane domain or the cytoplasmic domain is deleted.

15. A chimeric protein comprising the polypeptide of Claim 14 fused to a heterologous polypeptide.

16. The chimeric protein of Claim 15 in which the heterologous polypeptide is a constant region of an immunoglobulin.

17. An antibody that immunospecifically binds the Ob receptor protein of Claim 12 or 13.

18. An antibody that immunospecifically binds the polypeptide of Claim 14.

19. A method for diagnosing body weight disorders in a mammal comprising measuring obR gene expression in a patient sample.

20. The method of Claim 19 in which expression is measured by detecting mRNA transcripts of the obR gene.

21. The method of Claim 19 in which expression is measured by detecting the obR gene product.

22. A method for diagnosing body weight disorders in a mammal, comprising detecting an obR gene mutation contained in the genome of the mammal.

23. A method for screening compounds useful for the treatment of body weight disorders, comprising contacting a compound with a cultured host cell that expresses the obR gene, and detecting a change in the expression of the obR gene, a change in activity of the obR gene product expressed by the cultured cell, or a change in tyrosine phosphorylation of a host cell protein, or a change in ion flux in the host cell.

24. The method of Claim 23 in which expression of the obR gene is detected by measuring mRNA transcripts of the obR gene.

25. The method of Claim 23 in which expression of the obR gene is detected by measuring Ob receptor protein.

26. The method of Claim 23 in which tyrosine phosphorylation of host cell protein is assayed using an anti-phosphotyrosine antibody.

27. A method for treating a low body weight disorder in a mammal, comprising administering a compound to the mammal in an amount sufficient to inhibit activation of the Ob receptor by endogenous Ob.

28. The method of Claim 27 in which the low body weight disorder is anorexia, cachexia, bulimia, AIDS-related wasting or cancer-related wasting.

29. The method of Claim 27 in which the compound is delivered to the hypothalamus or the choroid plexus.

30. The method of Claim 27 in which the compound is an antagonist that binds to the Ob receptor and inhibits activation of the receptor.

31. The method of Claim 27 in which the compound binds to endogenous Ob and neutralizes Ob activity.

32. The method of Claim 31 in which the compound is a polypeptide corresponding to the extracellular domain of the Ob receptor or a portion of the extracellular domain that binds Ob, a deletion mutant Ob receptor protein lacking the transmembrane or cytoplasmic domain, or a chimeric fusion protein comprising the extracellular domain of the Ob receptor, or a portion of the extracellular domain that binds Ob, or a transmembrane deletion mutant fused to a heterologous polypeptide.

33. The method of Claim 32 in which the heterologous polypeptide of the chimeric fusion protein is the constant region of an immunoglobulin.

34. The method of Claim 32 or 33 in which the compound is delivered to the mammal by administering a genetically engineered host cell that expresses and secretes the polypeptide or fusion protein in the mammal.

35. The method of Claim 31 in which the compound is an anti-idiotypic antibody, or an Fab portion thereof,

that mimics the extracellular domain of the Ob receptor and neutralizes endogenous Ob.

36. A method for treating a low body weight disorder in a mammal, comprising administering a compound to the mammal in an amount sufficient to inhibit expression of the Ob receptor in vivo.

37. The method of Claim 36 in which the low body weight disorder is anorexia, cachexia, bulimia, AIDS-related wasting or cancer-related wasting.

38. The method of Claim 36 in which the compound is delivered to the hypothalamus or the choroid plexus.

39. The method of Claim 36 in which the compound is an antisense oligonucleotide that inhibits translation of mRNA transcripts that encode the Ob receptor.

40. The method of Claim 36 in which the compound is a ribozyme that inhibits translation of mRNA transcripts that encode the Ob receptor.

41. The method of Claim 36 in which the compound is an oligonucleotide that forms a triple helix with the regulatory region of the Ob receptor gene and inhibits transcription.

42. The method of Claim 36 in which the compound is a recombinant DNA construct that inactivates the Ob receptor gene or its regulatory region via targeted homologous recombination.

43. A method for treating a low body weight disorder in a mammal, comprising administering a compound

to the mammal in an amount sufficient to inhibit signal transduction induced by binding of endogenous Ob to the Ob receptor.

44. The method of Claim 43 in which the low body weight disorder is anorexia, cachexia, bulimia, AIDS-related wasting, or cancer-related wasting.

45. The method of claim 43 in which the compound is delivered to the hypothalamus or the choroid plexus.

46. The method of Claim 43 in which the compound inhibits the activity of an intracellular mediator of Ob receptor-induced signal transduction.

47. The method of Claim 46 in which the compound inhibits a tyrosine kinase or a tyrosine phosphatase.

48. The method of Claim 43, 44 or 45 in which the compound is an oligonucleotide construct encoding a signalling-incompetent Ob receptor controlled by a regulatory sequence that directs the expression of the signalling-incompetent receptor in target cells in the body.

49. The method of Claim 48 in which the oligonucleotide construct encodes a signalling-incompetent deletion mutant of the Ob receptor in which all or a portion of the cytoplasmic domain is deleted.

50. A method for treating obesity in a mammal, comprising administering a compound to a mammal in an amount sufficient to up regulate expression of a functional Ob receptor in the mammal.

51. The method according to Claim 50 in which the compound is delivered to the hypothalamus or the choroid plexus.

52. The method of Claim 50 or 51 in which the mammal expresses a defective Ob receptor and the compound comprises a nucleotide construct encoding a functional Ob receptor controlled by a regulatory region that directs expression of the functional receptor in target cells in the mammal.

53. The method of Claim 50 or 51 in which the mammal expresses a mutant Ob receptor and the compound comprises a nucleotide construct encoding a wild-type Ob receptor that corrects the endogenous mutation via targeted homologous recombination.

54. An isolated nucleic acid molecule encoding an Ob receptor, said nucleic acid molecule having a nucleotide sequence that encodes amino acids 1 to 868 of the amino acid sequence shown in Fig. 3.

55. An isolated nucleic acid molecule encoding an Ob receptor, said nucleic acid molecule having a nucleotide sequence that encodes amino acids 1 to 965 of the amino acid sequence shown in Fig. 3.

56. An isolated nucleic acid molecule encoding an Ob receptor, said nucleic acid molecule having a nucleotide sequence that encodes amino acids 1 to 1065 of the amino acid sequence shown in Fig. 3.

57. An isolated nucleic acid molecule encoding an Ob receptor, said nucleic acid molecule having a

nucleotide sequence that encodes amino acids 1 to 1115 of the amino acid sequence shown in Fig. 3.

58. An isolated nucleic acid molecule encoding an Ob receptor which can induce IL-6RE mediated gene expression.

59. An isolated nucleic acid molecule encoding an Ob receptor which can induce HRRE mediated gene expression.

60. The isolated nucleic acid molecule of any of claims 54-57, said molecule encoding an amino acid sequence having at least 90% identity to the amino acid sequence of Fig. 3.

61. The isolated nucleic acid molecule of claim 59, said molecule encoding an amino acid sequence having at least 90% identity to the amino acid sequence of amino acids 1 to 965 of Fig. 3.

62. A method for evaluating whether a test agent is a candidate agent for treatment of body weight disorders, comprising:

(a) exposing eukaryotic cells expressing a gene encoding an ObR polypeptide to said test agent; and

(b) measuring the expression of said gene by said eukaryotic cells in the presence of said test agent;

wherein said test agent is identified as a candidate agent for treatment of body weight disorders when the expression of said gene in the presence of said test agent differs from the expression of said gene by said eukaryotic cells in the absence of said test agent.

63. The method of claim 62 further comprising measuring the expression of said gene by said eukaryotic cells in the absence of said agent.

64. A method for evaluating whether a test agent is a candidate agent for treatment of body weight disorders, comprising:

(a) exposing eukaryotic cells expressing a gene encoding an ObR polypeptide to said test agent; and

(b) measuring the binding of said test agent to said eukaryotic cells;

wherein said agent is identified as a candidate agent for treatment of body weight disorders when the binding of said test agent to said eukaryotic cells differs from the binding of said test agent to control eukaryotic cells which do not express said gene, said control eukaryotic cells being otherwise identical to said eukaryotic cells expressing said gene.

65. The method of claim 64 further comprising measuring the binding of said test agent to said control eukaryotic cells.

66. A method for evaluating whether a test agent is a candidate agent for treatment of body weight disorders, comprising:

(a) exposing an obR polypeptide to said test agent; and

(b) measuring the binding of siad agent to said obR polypeptide;

wherein said test agent is identified as a candidate agent for treatment of body weight disorders when said test agent selectively binds said obR polypeptide.

67. The method of claim 62, 64, or 66 wherein said obR polypeptide comprises the cytoplasmic domain of obR.

68. The method of claim 67 wherein said cytoplasmic domain comprises amino acids 861 to 1165 of FIG. 3.

69. The method of claim 67 wherein said obR polypeptide further comprises the extracellular domain of obR.

70. The method of claim 66 wherein said obR polypeptide is fused to a detectable label.

71. The method of claim 62 or 64 wherein said eukaryotic cell is a choroid plexus cell.

72. The method of claim 62 or 64 wherein said gene is a recombinant gene.